Pesticides: An Important but Underused Model for the Environmental Health Sciences

Ernest Hodgson and Patricia E. Levi

Department of Toxicology, North Carolina State University, Raleigh, North Carolina

Pesticides are high-volume, widely used, environmental chemicals and there is continuous debate concerning their possible role in many chronic human health effects. Because of their known structures, known rates of application, and the presence of a large occupationally exposed population, they are not only important in their own right but are ideal models for the effects of environmental chemicals on the population in general. For reasons that are not always clear, this potential has not been realized. These exposed populations represent an underused asset in the study of the human health effects of environmental contaminants. Chronic effects thought to involve pesticides include carcinogenesis, neurotoxicity, and reproductive and development effects. In this paper we attempt to summarize this concern and, relying to a large extent on studies in our own laboratory, to indicate the importance and present status of studies of the mammalian metabolism of pesticides and indicate the need for further use of this model. Aspects considered include the role of pesticides as substrates for xenobiotic-metabolizing enzymes such as cytochrome P450 and the flavin-containing monooxygenase and their role as inducers or inhibitors of metabolic enzymes. The interaction of pesticides with complex multienzyme pathways, the role of biological characteristics, particularly gender, in pesticide metabolism, and the special role of pesticides at portals of entry and in target tissues are also considered. — Environ Health Perspect 104(Suppl 1):97–106 (1996)

Key words: pesticides, metabolism, oxidation, chronic effects, acute effects, cytochrome P450, flavin-containing monoxygenase, portals of entry, target tissues

Introduction

As a consequence of forces acting in different directions, it is difficult to foresee the changes that will take place in pesticide toxicology as they relate not only to human health but also to the environment. Since few efforts are now being made to moderate either national or world population growth, demands for food and fiber must increase, bringing into use marginal land and marginal climatic zones. At the same time compounds useful in the production of food and fiber are lost both to pest resistance and to regulatory action. While the cost of developing new pesticides has dramatically increased, which limits such development to a small number of companies, new compounds are appearing along with questions concerning their potential for human and environmental risk. Integrated pest management, when successful, can limit the amounts of pesticides needed, but in the foreseeable future, it is unlikely to do more than slow the rate of increase in the use of these chemicals.

It is clear that pesticide toxicology is of importance in its own right, involving numerous chemicals designed to be toxic to one or more living organisms and released intentionally into the environment. At the same time, their role as model compounds for environmental chemicals in general should not be forgotten. Unlike many environmental chemicals such as combustion products, industrial waste, etc., the chemical structures of pesticides are known and they are released intentionally in amounts that are either known or can be determined. Furthermore, pesticide manufacturing workers, formulators, applicators, and agricultural workers and their families constitute a body of individuals exposed to relatively high concentrations of pesticides. Since these individuals are exposed in the course of normal, usually legal, use of pesticides, the ethical problems involved in administration of chemicals to human study groups are avoided. Studies of these groups should be an essential preliminary to consideration of the risk to the general population of the much smaller concentrations of pesticides to which they are exposed.

Despite these needs and advantages, pesticides remain relatively unexplored either as important potential toxicants in their own right or as models for environmental toxicants in general. Investigators in the biochemical and molecular aspects of environmental toxicology frequently use model compounds that are either clinical drugs with little if any environmental significance or convenient chemicals, again with little or no environmental significance. In any case it remains true that in even in metabolic studies in experimental animals the isozyme specificity for pesticides, whether they are acting as substrates, inhibitors, or inducers, is largely unknown. Further, in only a vanishingly small number of cases has the role of human isozymes in pesticide metabolism been examined. The remainder of this communication is devoted to highlighting several aspects of the current state of knowledge of the interaction of pesticides with living organisms, primarily at the biochemical and molecular levels. Since space considerations preclude a detailed review of the subject, considerable emphasis is on the contributions of the authors and their associates in the area of molecular and biochemical interactions of pesticides. Knowledge of such interactions is essential for understanding mechanisms of toxicity and development of realistic risk assessment models.

Pesticides (*1–5*) are metabolized by many enzymes, including the cytochrome P450-dependent monooxygenase system (P450), the flavin-containing monooxygenase (FMO), prostaglandin synthetase, molybdenum hydroxylases, alcohol and aldehyde dehyrogenases, esterases, and a

Manuscript received 28 August 1995; manuscript accepted 12 December 1995.

Address correspondence to Dr. Ernest Hodgson, Department of Toxicology, Box 7633, North Carolina State University, Raleigh NC 27695. Telephone: (919) 515-5295. Fax: (919) 515-7169. E-mail: Ernest_Hodgson@ncsu.edu

Abbreviations used: FMO, flavin-containing monooxygenase; DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; DDE, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethylene; NADPH, reduced nicotinamide adenine dinucleotide phosphate; MDP, methylenedioxyphenyl; PBO, piperonyl butoxide; SES, sesamex; ISO, isosafrole; SAF, safrole; TDZ, thioridazine; IPPSF, isolated perfused porcine skin flap; ABT, aminobenzotriazole.

variety of transferases, most notably the glutathione *S*-transferases. Involved in what is usually the initial metabolic attack on the pesticide, P450 appears to be the most important, followed by the FMO. Depending upon the pesticide substrate, examples of both activation and detoxication can be found with any of these enzymes; however, P450 isozymes have added importance as activation enzymes, producing reactive electrophiles that interact with nucleophilic substituents on macromolecules such as proteins and nucleic acids. Pesticides serve not only as substrates for these enzymes but, particularly in the case of P450, may serve also as inhibitors or inducers. These multiple roles are illustrated by studies on such pesticides as the methylenedioxyphenyl synergists, organophosphates, organochlorines, and herbicide synergists (*5–8*).

Over 400 P450 genes have been characterized and their nucleotides and derived amino acid sequences compared. In a number of cases, the genes have been mapped to specific chromosome loci and in others the mechanism of expression has been investigated. A system of nomenclature based on derived amino acid sequences was proposed in 1987 and updated in 1989, 1991, and 1993. P450 genes are designated *CYP* (the protein products may still be designated P450) followed by a numbering system that distinguishes gene families, gene subfamilies, and individual genes. This system has enabled an evolutionary tree for P450s to be developed (*9*). While some P450s are substrate specific, those involved in xenobiotic metabolism tend to be relatively nonspecific, although, even in the latter, substrate preferences are usually evident. Isozyme specificities also exist for inhibitors and inducers of P450 isozymes.

FMOs, like P450s, are located in the endoplasmic reticulum of vertebrate cells and are involved in the monooxygenation of pesticides. Originally described as an amine oxidase (*10*), FMOs are now known to catalyze the oxidation of many organic, and some inorganic, chemicals (*11,12*). They are particularly important in the oxidation of heteroatoms, particularly nitrogen, sulfur, phosphorus, and selenium in organic molecules.

Pesticides as Human Health Hazards

Acute

Toxic outbreaks or collective poisonings have resulted from misuse of almost every type of pesticide: organochlorine insecticides such as DDT, lindane, and chlordecone; chlorinated camphenes such as toxaphene; the cyclodienes aldrin and dieldrin; organophosphate and carbamate cholinesterase inhibitors; organomercury fungicides; inorganics; and others. Such collective outbreaks may be defined as the effect, in an exposure incident, of a chemical or group of chemicals on a population in which several to many individuals are poisoned. Collective toxic outbreaks related to pesticides have recently been the subject of a comprehensive review (*13*). They may occur in the general population from oral or cutaneous exposure or they may be occupational in nature, involving workers in manufacturing or formulators, mixers, or applicators in agriculture and public health. While it is clear that such incidents can occur in any country, in recent years they have become less common in developed countries than in developing countries. Emergency response time and therapy also tend to be shorter and more effective, respectively, in more developed countries, so that it might be expected that this trend will continue.

While reliable statistics on individual poisonings by pesticides are more difficult to obtain, it appears that again the same trend toward a less serious situation in developed countries is occuring. It is interesting to note that many pesticide-related deaths seem to involve suicide. At the same time there is concern (*14*) that there may be serious sequelae following apparent recovery after treatment from an acute poisoning episode, particularly in the case of organophosphate poisoning or chronic, noncholinergic effects from the same compounds.

Chronic

Recently, public concern over potential adverse health effects has focused on a number of chronic end points—carcinogenesis, developmental and reproductive effects, immunological effects, and neurotoxicity. Moreover, the mechanisms by which environmental chemicals, not only pesticides, can contribute to these chronic events are still largely unknown. Thus, adequate test protocols are still generally unavailable or not yet in use.

It is seems apparent, by extrapolation from hazard assessment studies conducted primarily in rodents, that pesticides have the potential to produce toxicity in humans, a potential that includes many different toxic end points [see Baker and Wilkinson (*15*), for a summary]. While this potential almost certainly occurs in the occupational setting, it is more difficult to confirm for the general population, exposed primarily through the diet (food and drinking water). Epidemiology studies almost always involve occupational exposures and, even then, are complicated by multiple sequential exposures. Extrapolation from rodent assays to the levels in food and drinking water, often at or about the limit of detection, is the broadest possible extrapolation and thus the most subject to error. It is important that future studies properly define the chronic toxicity of low doses of pesticides to the human population. Moreover, given the generally low incidence of toxic effects, direct evidence of toxic mechanism or, at least, a plausible explanation based on mechanistic toxicology will be necessary. The consequences from positive correlations are serious and regulation will be necessary, while the consequences from negative correlations could save society from needless cost or panic-induced regulations.

Carcinogenesis. In recent years, there has been an increse in public concern that chronic low-level exposure to pesticide residues in food and water might pose a serious cancer risk to the general population. While epidemiological studies have often implicated pesticides as causative agents in human cancer (*16–20*), it has usually been at a marginal level of significance. In the past, both experimentalists and epidemiologists have generally thought of chemical carcinogens as electrophiles and mutagens which act as initiating agents through genotoxic mechanisms. Today, however, we realize that chemicals can play a role in the cancer process by a number of nongenotoxic mechanisms such as promotion, peroxisome proliferation, hormone imbalance, and cytotoxicity leading to compensatory cell division (mitogenic agents). It is likely that many pesticides, classified as animal carcinogens, are acting through one of these mechansims. For example, it is suspected that 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) and its breakdown product 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE), still persistent in the environment long after being banned, may be involved in the causation of breast cancer as a result of estrogenic activity (*21–25*). Again, it is essential that this issue be resolved. The marginal levels of significance in most epidemiological studies of pesticides has led to a general suspicion that pesticides may

often be promotors, rather than initiators, of cancer. That this is possible is clear from the mechanistic studies by Smart and his group (*26–29*) into the tumor-promoting effects of mirex. Not only is mirex a potent tumor promotor in the mouse skin model, but it has a mechanism of action different from that in classic promoters, such as the phorbol ester, TPA. Studies are ongoing to define this novel mechanism that may be applicable, not only to pesticides, but to other environmental contaminants as well.

Neurotoxicity. Because of the basic similarities between mammalian and insect nervous systems, insecticides designed to attack the insect nervous system (organochlorines, organophosphates, and carbamates) are capable of producing acute and chronic neurotoxic effects in mammals (*14,30,31*). In fact, both acute and chronic alterations in sensory, motor, autonomic, cognitive, and behavioral functions have been observed in people exposed occupationally to relatively high levels of insecticides and other pesticides. While workers exposed occupationally to pesticides constitute an extremely large workforce worldwide, they have received little attention with respect to the possible occurrence of adverse neurological effects. If neurotoxic effects are not readily observed in this population, it is unlikely that they would be detected in the general population where pesticide exposure is only at trace levels. While our knowledge of acute neurotoxic effects of pesticides and other environmental chemicals is fairly extensive, our current understanding of chronic effects is considerably less extensive. To rigorously evaluate chronic neurotoxic effects, we need more sound epidemiological and experimental data.

Reproductive Effects. A number of pesticides clearly have the potential to cause reproductive toxicity in animals, and several (e.g., dibromochloropropane [DBCP], ethylene dibromide [EDB], chlordecone [Kepone], and carbaryl) are known to affect human reproduction (*32–37*). Reproductive toxicants have the potential to cause adverse effects by one of several mechanisms. Some are direct-acting agents, either through their chemical reactivity (e.g., germ cell destruction by ionizing radiation) or by structural similarity to endogenous molecules (e.g., hormone agonists or antagonists). Other xenobiotics can interrupt reproduction indirectly either by metabolism to a direct-acting toxicant or by altering the endocrine system (e.g., increased steroid clearance).

One important mechanism of action of reproductive toxicants results from structural similarlty of the parent compound or a metabolite to an important biological molecule such as a hormone. Xenobiotics in this category then act as agonists or antagonists of the endogenous hormones. Since the early 1970s, it has been known that fetal exposure to DDT causes male animals to develop abnormally small penises and undescended testicles. For many years these effects were thought to result from the estrogenic action of DDT. Recent studies (*38*), however, demonstrate that the primary DDT metabolite *p,p'*-DDE, interferes with the action of male sex hormones, or androgens, suggesting that the feminizing action of DDT resulted from the antiandrogenic action of the DDT metabolite. Has exposure to DDT and other hormonally active pesticides played a significant role in the reported increased incidence of male reproductive abnormalities in humans, such as the the reported decline in sperm counts and the rise in testicular cancer? Clearly this awaits further investigation.

Developmental Effects. Traditionally, mammalian developmental toxicity has referred to adverse effects initiated or evident during *in utero* development. More broadly, however, developmental toxicity also includes adverse effects on the developing organism that may have resulted from exposure of either parent before conception, of the mother during prenatal development, or postnatally to the time of sexual maturation. Development is an exceedingly complex process and the sensitivity of the conceptus to chemicals, as well as the type of abnormality expressed, varies with the developmental stage at which exposure occurs. The embryo is most vulnerable to the initiation of major birth defects between 3 weeks and 2 months of gestation, the critical period of organogenesis. Exposure to toxic chemicals during the first 2 weeks typically leads to fetal death, while exposure after organogenesis is more likely to cause growth retardation and functional deficits.

Data currently available are inadequate to make a meaningful estimate of the degree to which pesticide exposure may be involved in human developmental problems. Animal data clearly indicate that the potential is there, provided exposure occurs during sensitive time points and at sufficient concentrations. Of particular current concern is exposure to hormonally active chemicals, including pesticides. In humans and rodents, exposure to hormonally active chemicals during the period of sex differentiation can produce a wide range of abnormal sexual phenotypes including masculinized and defeminized females and feminized and demasculinized males. Xenobiotics known to act in this capacity have been referred to as environmental estrogens or environmental androgens. As mentioned in the discussion above on reproductive effects, compounds structurally similar to hormones may act either as receptor agonists or antagonists. Pesticides possessing hormonal activity and having the ability to interfere with mammalian sex differentiation include methoxychlor (*39,40*), DDT (*38*), and vinclozin (*41*).

The Significance of Pesticide Metabolism

Pesticides as Substrates

P450 carries out many different monooxygenations of pesticide substrates, such as epoxidation (e.g., aldrin), *N*-dealkylation (e.g., atrazine), *O*-dealkylation (e.g., chlorfenvinphos), *S*-oxidation (e.g., phorate), and oxidative desulfuration (e.g., parathion) (*8*). Substrates for the FMO are similarly diverse but all are soft nucleophiles, a category that includes many organic chemicals with sulfur, nitrogen, phosphorus, or selenium heteroatoms. Although xenobiotic-metabolizing isozymes of P450 appear to prefer hard nucleophiles as substrates, there is considerable overlap and most, if not all, substrates for FMO are also substrates for P450. The reverse, however, is not true since oxidations at carbon atoms are readily catalyzed by P450 but rarely, if at all, by FMO. However, even when the same substrate is oxidized by both FMO and P450, there may be differences in the rate of oxidation, in the products, or in the stereochemistry of the same product. The concentration of various isoforms of both FMO and P450 varies from tissue to tissue. Pesticide substrates for the FMO include organophosphates such as phorate and disulfoton, which yield sulfoxides; the phosphonate fonofos, which yields fonofos oxon; carbamates such as aldicarb and methiocarb; dithiocarbamate herbicides such as sodium metham; botanical insecticides such as nicotine; and cotton defoliants such as the trivalent organophosphorus compound folex (*42–45*).

Very little information on the contribution of individual isozymes of either P450 or FMO to pesticide oxidations is

available. Early studies in this laboratory using partially purified P450 enzyme preparations from uninduced mouse liver showed considerable variation in oxidation of pesticide substrates (*46*) and in interactions (spectral binding and inhibition of activity) with the pesticide synergist piperonyl butoxide (*47*).

Later studies (*48*) using highly purified P450s from phenobarbital-induced mouse livers (CYP2B) and from β-naphthoflavone-induced mouse livers (CYP1A) showed that these induced isozymes possessed much higher levels of activity toward the organophosphates fenitrothion, parathion, and methyl parathion than did the P450 fractions purified from untreated mice, thus suggesting the importance of these P450 families in pesticide metabolism. In addition to the high level of activity, the isozymes produced different ratios of oxon (activation) to phenol (detoxication), with CYP2B forming more of the activation product than CYP1A. Similar differences in activation to detoxication ratios were found in P450 fractions from the uninduced livers. Thus the amount of toxic metabolite formed would be a function of the isozyme composition of the animal.

Studies with phorate using microsomal preparations (*49,50*) showed very high levels of phorate sulfoxidation in microsomal preparations from phenobarbital-induced mouse livers (primarily CYP2B) compared to untreated or acetone-induced mouse livers (induction of constitutive CYP2E), thus supporting the importance of the inducible CYP2B forms in pesticide (xenobiotic) oxidations.

Recently Rose et al. (unpublished data) have determined the activity of human liver microsomes and some expressed human P450 isoforms toward pesticides. The activity of human liver microsomes toward ethoxyresorufin, phorate, and parathion was about 10% of the activity of mouse liver microsomes toward these same substrates, although hydroxylation of *p*-nitrophenol was similar for both species. The human isoforms were expressed either in human lymphoblastoid cell lines (Gentest Corp., Woburn, MA) or, in the case of those from the P4502C family, in yeast by J. A. Goldstein. P450s 1A2, 2E1, 3A4 and members of the 2C family are all capable of metabolizing phorate to phorate sulfoxide, although activity varies considerably between isoforms.

Although parathion was metabolized by human liver microsomes in a reaction requiring reduced nicotinamide adenine dinucleotide phosphate (NADPH), the expected product, paraoxon, was not observed; only *p*-nitrophenol was detected. Further studies revealed that paraoxon, on incubation with human liver microsomes, with or without NADPH, was rapidly hydrolyzed to *p*-nitrophenol by microsomal esterases, suggesting that paraoxon formed by the P450-mediated oxidative desulfuration of parathion is immediately hydrolyzed to *p*-nitrophenol. This result emphasizes the potential importance, in organophosphate poisoning, of the small amount of oxon formed at the site of action (*51*) compared to the larger amount formed in the liver that may never reach the site of action.

Expression of Pesticide-metabolizing Enzymes

Pesticides as Inducers

A number of studies have provided evidence for induction of liver enzymes in humans exposed occupationally or environmentally to pesticides based on half-life of aminopyrene or phenylbutazone or excretion of 6β-hydroxycortisol (*52–56*). Numerous early experiments with laboratory rodents confirmed hepatic enzyme induction (*7*), although at the time, methods were not available for identification of individual isozymes. Generally, induction of microsomal enzyme activities were assessed either *in vivo* or *in vitro* and the pesticide was often classified a phenobarbital, a 3-methylcholanthrene, or a mixed-type inducer.

1,1,1-Trichloro-2,2-bis(p-chlorophenyl)ethane (DDT). Work by Abernathy et al. (*57,58*) demonstrated significant decreases in zoxazolamine paralysis time, hexobarbital sleeping time, and aniline hydroxylase activity in mice following treatment with DDT or DDE, a major metabolite of DDT and an important contaminant of animal fat.

Mirex and Chlordecone. Previous studies in our laboratory (*59*) demonstrated the induction of CYP2B10 protein and associated enzymatic activities by acute exposure to both mirex and chlordecone (Kepone). Subsequently Adams et al. (unpublished data) showed that chronic low-level dermal application of mirex also induced CYP2B10 in mouse liver. The enzymatic activities measured in these studies suggested that, in addition to 2B10, other P450(s) were induced. We have recently demonstrated the induction of two P450s in addition to 2B10, namely 1A2 and 3A. It is of interest that 1A2 and 3A forms are constitutively expressed in both human and mouse liver. Since CYP 3A3/4 is one of the major forms in human liver and is involved in steroid hydroxylation, we have initiated studies in conjunction with G. LeBlanc to determine the effect of mirex induction on testosterone hydroxylation in both male and female mice. These studies reveal a significant increase in the total amount of hydroxylated metabolites produced and also in the relative amounts of several products within that total, with male mice being more affected than female. This increased metabolism of testosterone is accompanied by a decrease in circulating levels of serum testosterone. Exactly what the implications are for this alteration in hormone levels is still to be determined.

Methylenedioxyphenyl Compounds. Piperonyl butoxide (PBO) and sesamex (SES) have been used as synergists with pyrethroid and carbamate pesticides, and isosafrole (ISO) and safrole (SAF) are found in many common foods of plant origin, with SAF having been shown to be a liver carcinogen in rodents at high doses. Methylenedioxyphenyl (MDP) compounds affect multiple enzyme pathways (*60,61*), including the P450 monooxygenase system. The effect of MDP compounds on P450 activity is biphasic, with an initial inhibition of activity followed by an increase above control levels (*50,62,63*). The inhibitory effect of MDP compounds has been attributed to the formation of a stable inhibitory metabolite complex between the heme iron of the P450 and the carbene species formed when water is cleaved from the hydroxylated methylene carbon of the MDP compound (*64*). MDP exposure induces several P450 isozymes not found in detectable quantities in unexposed animals (*65–68*).

Several studies have been published regarding the effects of MDP compounds on mammalian liver enzymes [for review, see (*68*)]. Cook and Hodgson (*69*) showed that ISO increased the level of Ah receptor in mice but did not displace receptor-bound 3-methylcholanthrene (3MC) or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), both of which interact with the Ah receptor to induce P450s. Cook and Hodgson (*70*) also demonstrated that there was comparable induction of P450 in a congenic strain of C57 mice which lacked a functional Ah receptor and in Ah receptor proficient C57 mice. MDP compounds have been reported to induce P450 isozymes CYP1A2 and CYP2B10 in the mouse

(*65*). Some investigators have reported that CYP1A1 is also induced in mice by MDP compounds (*71*). In induction studies in rats using 4-*n*-alkyl MDPs, the length of the alkyl side chain affected which P450 isozymes were preferentially induced, with the six-carbon side chains favoring 2B1; the rat P450 was most similar to mouse 2B10 (*71*). In another study, MDP compounds with electron-donating side chains were reported to be P450 inducers while MDP compounds with electron withdrawing groups were not (*72*).

Regulation of cytochrome P450 isozymes 1A1, 1A2, and 2B10 by MDP compounds was studied in our laboratory by measuring mRNA and protein levels, as well as enzyme activities in hepatic tissue, from C57BL/6 (Ah^+) and DBA/2 (Ah^-) mice dosed with ISO or PBO (*66–68*). Increases in 1A2 and 2B10 were observed for ISO and PBO in both stains of mice, suggesting an Ah receptor-independent mechanism for induction of these isozymes; 1A1 induction, however, was seen only in C57 mice and only at high doses of PBO. Dose–response studies showed maximum inducible levels for 1A2 and 2B10 protein, beyond which the mRNAs continued to increase while the protein levels remained constant.

Further studies of the induction of the P450 isozymes 1A1, 1A2, and 2B10 were carried out (*66–68*) using four MDP compounds (SAF, ISO, PBO, and SES) and the non-methylenedioxyphenyl analog of SAF, allyl benzene, in male C57BL/6N mice. CYP1A1 was not detected in control animals and was induced by SES and PBO, with SES inducing higher levels of 1A1 protein than PBO. CYP1A2 mRNA was detected in the livers of control animals and was increased by all MDP compounds (SES > PBO ≅ ISO > SAF). Allyl benzene treatment did not induce detectable levels of 1A1, 1A2, or 2B10, suggesting that the methylenedioxy moiety is important in induction.

Recent studies (*73*), using the closely related benzodioxoles 5-*t*-butyl-1,3-benzodioxole; 5-*n*-butyl-1,3-benzodioxole; and 5-(3-oxobutyl)-1,3-benzodioxole, have further defined the involvement of this class of compounds in the regulation of P450 isozymes, confirming that even in the Ah^+ C57BL/6 strain of mice, none of these compounds induced CYP1A1. All three benzodioxoles induced protein and mRNA message for CYPlA2, while the *t*-butyl analog also induced both protein and message for CYP2B10.

Thus all of the studies of the effects of methylenedioxyphenyl compounds on cytochrome P450 in mice suggest that CYP1A2 can be induced by a non-Ah receptor-dependent mechanism as well as by an Ah-dependent mechanism. This is currently being explored.

Tridiphane. The herbicide synergist tridiphane [2-(3,5-dichlorophenyl)-2-(2,2,2-trichloroethyl) oxirane] is a postemergent herbicide used in conjunction with atrazine, its activity being attributed to its ability to inhibit glutathione *S*-transferases. Tridiphane is also known to be a peroxisome proliferator and to induce epoxide hydrolase in rodents (*74*).

Tridiphane is an excellent example of a pesticide that can function both as an inhibitor and an inducer of cytochrome P450, with different isozymes specificities for each activity. Induction of CYP4A protein and associated enzyme activity has been demonstrated (*75*). The CYP4A enzymes are constitutive proteins known to be involved in the ω-hydroxylation of medium- and long-chain fatty acids. The importance of peroxisome proliferation in human health has yet to be clearly defined because many aspects of this phenomenon appear to be rodent specific. Nevertheless, until the effect has been defined, it is not something that should be ignored.

Endogenous Effects

Many studies have demonstrated that the oxidation of xenobiotics, including pesticides, can be affected by both endogenous and exogenous factors. Endogenous factors include species, strain, age, gender, and hormonal status while the exogenous factors include such factors as stress and diet. Most of these studies have been carried out on reactions mediated by the cytochrome P450-dependent monooxygenase system and, in the case of pesticides, have seldom been carried out on individual isoforms either at the level of protein or mRNA expression. Virtually nothing is known of the role of exogenous or endogenous factors on the metabolism of pesticides by FMO.

Gender Effects. Recently (*76*) we have examined the role of gender in the expression of FMO isoforms in mouse liver. While it has long been known that the FMO activity toward several substrates was higher in the liver of female than of male mice, these studies were carried out at the level of substrate oxidation and before it was known that several different isoforms of FMO exist in mammals. Hepatic FMO activity of microsomes from adult CD-1, Swiss-Webster, C57BL/6, and DBA/2 mice was found, in all cases, to be significantly higher in females than in males. Based on protein and mRNA levels in CD-1 mice, it was shown that the isoforms responsible for this difference were FMO1 and FMO3; FMO5 was the same in the livers of mice of either gender. FMO1 was 2 to 3 times higher in female liver than in male liver while FMO3, expressed at levels similar to those of FMO1 in females, was not detected in the liver of males. There was close correlation between protein levels and mRNA levels in each case. Thus, in mouse liver there is a gender-independent isoform (FMO5), a gender-dependent isoform (FMO1), and a gender-specific isoform (FMO3). Neither FMO2 nor FMO4 was detected in the liver of mice or either gender. This effect, while dramatic, is tissue dependent. FMO1, FMO3, and FMO5 are expressed at similar levels in the lung and kidney of CD-1 mice of both genders.

Pesticides as Inhibitors

There are several mechanisms by which pesticides may act to inhibit P450 enzymes: competitive inhibition, whereby the pesticide competes with the substrate at the active site, thus preventing substrate binding (the pesticide may or may not also be a substrate); noncompetitive inhibition in which the pesticide binds to the enzyme in such a way as to alter the activity of the enzyme toward its substrate; and suicide inhibition, whereby the pesticide is metabolized by the enzyme and a metabolite of the pesticide binds to the enzyme, irreversibly inhibiting its activity.

As mentioned earlier, MDP compounds have very complex interactions with the P450 system (*50,60–73*). Since MDP compounds are substrates for P450 enzymes, they may act initially as competitive inhibitors. *In vitro* with microsomal systems, this interaction is manifested as a type I binding spectrum with a peak at 390 nm and a trough at 420 nm. As the MDP compound is metabolized, it becomes a suicide inhibitor, which as a reactive metabolite (probably a carbene) forms a stable inhibitory complex with the heme iron of P450. It is this latter characteristic that is responsible for the synergistic activity of these compounds, most notably piperonyl butoxide, in pesticide formulations.

As noted above, the herbicide synergist tridiphane is a postemergent herbicide, and its activity is attributed to its ability to inhibit glutathione *S*-transferases.

Tridiphane has been shown to induce peroxisome proliferation, epoxide hydrolase (*74*), and P450 (*75*) in rodents. In addition to induction of CYP4A, tridiphane functions as a selective P450 inhibitor, inhibiting CYP2B10 while having little or no effect on other P450 isozymes (*77*). As assessed by *in vitro* studies, tridiphane appears to be a competitive inhibitor of the P450 enzymes; its effect *in vivo,* however, is not yet known.

Organophosphorus insecticides such as parathion that contain the P = S moiety are metabolized by the P450 system to the corresponding oxon, P = O, by oxidative desulfuration. This activation reaction, which converts the relatively inactive compound to a potent cholinesterase inhibitor, is thought to involve the formation of a P-S-O (phosphooxythirane) ring intermediate. Studies with both microsomes and purified enzymes (*78–80*) have demonstrated that, during oxidative desulfuration, the released sulfur exists as a highly reactive molecule which then binds to P450, inactiviting the enzyme. This binding of reactive sulfur to P450 is accompanied by loss of P450 as detected by measurement of the dithionite-reduced CO complex as well as loss of monooxygenase activity (*81–84*). Studies in our laboratory with purified P450s and the pesticide fenitrothion demonstrated that the amount of inhibition varied with the P450 isozyme, with CYP2B being inhibited more than CYP1A (*48*).

Pesticides in Complex Multienzyme Pathways

General Approach

While P450 and FMO have many substrates in common, the products of these substrates may be different and have different toxic potencies. Thus, it is important to know the relative contribution of the two pathways to the metabolism of a particular substrate. Furthermore, in contrast to FMO isozymes, xenobiotic-metabolizing isozymes of P450 are often relatively easily induced, thus making the relative contributions variable with the conditions of exposure. Although it is said that the FMO prefers soft nucleophiles as substrates and P450 hard nucleophiles, with the exception of compounds oxidized at carbon atoms, this applies only to the relative ability of compounds to serve as substrates for one or the other because it is difficult to find FMO substrates that are not also substrates for one or more P450 isozymes.

Such substrates may have complex oxidation patterns involving both FMO and P450 isozymes and show different regioselectivity in the sites on the molecule attacked. As a result, these substrates may yield different products or different isomers of the same product. A number of methods are available for determining the relative contributions of FMO and P450 including extrapolation from the properties of purified enzymes (or from isozymes cloned and expressed in expression systems), the use of product-specific substrates, the use of enzyme-specific substrates, or the manipulation of microsomes in which both enzymes are found. This latter technique, using heat treatment to inactivate FMO or an antibody to the NADPH-P450 reductase to inactivate P450, has proven most useful in our hands, particularly in the case of hepatic enzymes (*42,49,50,85*). At least one of the FMO forms found in the lung and kidney is resistant to thermal interaction. This form, however, is not expressed in liver.

Phorate. The insecticide phorate undergoes a complex series of oxidations. The products are generally more toxic than phorate and the reaction sequence is, therefore, an activation sequence. This substrate has proven to be useful in examining the relationship between FMO and P450 activity.

FMO forms only one product, phorate sulfoxide, while P450 yields phorate sulfoxide as well as additional products. Moreover, the sulfoxidation reaction is stereospecific with FMO producing the (–) sulfoxide and several P450 isozymes the (+) sulfoxide. While both sulfoxide isomers are substrates for further metabolism by all P450 isozymes tested, the (+) sulfoxide is always preferred to the (–) sulfoxide (*45*). The relative contribution of FMO to sulfoxide formation is higher in female than in male mice, in agreement with the studies of gender effects described above. Although overall sulfoxide formation is higher in the liver than in any extrahepatic tissue, the contribution of the FMO relative to P450 is higher in lung, kidney, and skin—being as high as 90% of the total metabolism in renal microsomes from the female mice. By contrast in the liver, P450 is responsible for more of the phorate sulfoxidation (~50–70%, depending upon the gender). Furthermore, the contribution of P450 relative to FMO is increased following treatment *in vivo* with inducers of hepatic P450 such as phenobarbital (*49,50*).

Target Tissues

Nervous System. Initial studies in this area in our laboratory involved the antipsychotic drug thioridazine (TDZ). They are currently being extended to include pesticide substrates. Many drugs, including antipsychotics, monoamine oxidase inhibitors, and antihistamines, are substrates for the FMO; TDZ is an excellent substrate for examining the relative importance of different oxidative pathways because it is oxidized at multiple sites by both FMO and P450.

Based primarily on examination of urinary and serum metabolite profiles, S-oxidation appears to be the predominant route of TDZ, metabolism in humans by producing the 2-sulfoxide, the 2-sulfone, and the 5-sulfoxide. The 2-sulfoxide and the 2-sulfone are known to have greater antipsychotic activity than the parent compound, while the ring sulfoxides may be responsible for the cardiotoxic side effects sometimes seen with TDZ [see Hodgson et al. (*4,5*) for review].

Metabolism by hepatic microsomes from the mouse yielded primarily the 2-sulfoxide of TDZ, with significant amounts of the 5-sulfoxide, the *N*-oxide, the *N*-demethyl derivative, and the 2-sulfoxide-*N*-oxide (*86*). Heat treatment of microsomes to selectively destroy the FMO activity or treatment with an antibody to the NADPH–cytochrome P450 reductase to inhibit P450 isozymes revealed that the *N*-oxide was the principal metabolite derived from the FMO, while the 2-sulfoxide and the other products were derived primarily from one or more isozymes of cytochrome P450.

Studies using FMO purified from mouse liver have shown the *N*-oxide of TDZ to be the principal and perhaps the only product of TDZ metabolism by this enzyme. Similar experiments using P4502D6 revealed that the 2-sulfoxide of TDZ (mesoridazine) is the principal but not the only metabolite of TDZ with this P450 isozyme. Similar experiments have been carried out with P450 isozymes 2E1 and 2B1.

The possible occurrence and role of FMO in the nervous system appears to be important not only because TDZ and several of its metabolites have similar biological properties and activation within the target tissue may be significant but also because the FMO has been shown to activitate phosphonates by metabolism to their oxons (*43,87,88*) and to carry out the sulfoxidation of phorate and other

insecticides. Previous studies from other laboratories that indicate the possible occurrence of the FMO in rat corpus striatum and whole brain microsomes could not be duplicated. Studies in our laboratory, using microsomes prepared from mouse brain, of substrate level oxidations and western blotting with an antibody to a form of FMO purified from mouse liver, while not negative, were equivocal. To determine the presence of FMO mRNA in rabbit brain, we, in conjunction with R.M. Philpot, have recently used PCR techniques to demonstrate FMO in the nervous system of the rabbit (Blake et al., unpublished data). Recently, five forms of FMO (forms 1 through 5) have been identified in rabbit hepatic and extrahepatic tissues (*89–91*), with most tissues expressing more than one form. PCR amplification of cDNA was performed using primers specific for each of the five forms of FMO found in rabbit tissues. These data suggest that only one form, apparently FMO4, is expressed in rabbit brain. The cDNA for this FMO has been cloned and sequenced from a human liver cDNA library (*92*).

The substrate specificity of FMO4 has not been determined. If it is a metabolically active protein, the inability to detect its presence in brain is probably due to its localization in certain brain regions or cell types. Studies are in progress to confirm the presence of FMO4 message in brain and to use immunocytochemical and *in situ* hybridization techniques to localize the isozyme in the brain.

Portals of Entry

Skin. Because the skin is continuous over the large surface area of the body and is in direct contact with the environment, it is often the portal of entry for pesticides and other environmental contaminants, as well as the site of transdermal absorption of clinical drugs. Thus, knowledge of the enzymes involved in xenobiotic metabolism by skin is important for understanding mechanisms of toxicity and for developing more realistic risk assessment models (*93*). The skin is known to contain many of the xenobiotic metabolizing enzymes found in the liver, including P450, transferases, esterases, and epoxide hydrolase (*94–98*). Moreover, P450 activity in skin has been shown to be induced by polycyclic aromatic hydrocarbons such as 3-methylcholanthrene and other xenobiotics (*99–103*).

We have recently studied pesticide substrates in mouse skin (*44*). In these studies the roles of P450 and FMO were studied in skin microsomes and compared to those in liver. The P450 content of skin was approximately 6.8% of the liver P450 content. By comparison, NADPH–cytochrome c reductase activity in skin microsomes was high—approximately one-third of the liver microsomal enzyme activity. Skin microsomes metabolized several known P450 substrates and, depending upon the substrate used, the specific activity ranged from 2.5 to 13.4% of the corresponding rates seen in liver microsomes. Skin microsomes exhibited the highest enzymatic activity with benzo[*a*]pyrene and ethoxyresorufin, moderate activity with parathion and aldrin, and low activity with benzphetamine and ethoxycoumarin. Skin microsomes also metabolized the triazine herbicides atrazine, simazine, and terbutryn, with the activity being 2 to 5% of the liver microsomal activity. FMO activity in skin microsomes with thiobenzamide and methimazole as substrates ranged from 10 to 20% of the liver FMO activity. Immunohistochemical studies using antibodies to mouse liver FMO showed localization primarily in the epidermis. Additional studies using pig skin showed a similar distribution pattern. Antibodies developed to mouse liver FMO and the constitutive liver P450 isozyme CYP1A2 showed cross-reactivity on Western blots; proteins in skin microsomes appeared identical to the cross-reacting protein present in liver microsomes. The relative contribution of P450 and FMO in mouse skin to the sulfoxidation of phorate was investigated and compared to that of liver microsomes. Several procedures were employed to selectively inhibit either P450 or FMO so that the role of each monooxygenase system, in the absence of the other system, could be determined. As in the lung and kidney, FMO in the skin proved to be relatively more important than P450 for the sulfoxidation of phorate. In liver microsomes, P450 was responsible for 68 to 85% of the phorate sulfoxidation activity. In contrast in skin microsomes, 66 to 69% of the phorate sulfoxidation activity was due to FMO, while P450 was responsible for the remainder of the activity. Although the overall phorate sulfoxidation rate in mouse skin microsomes was only 3 to 4% of the rate seen in liver, FMO appears to assume a greater role relative to P450 in the metabolic processes in skin.

While microsomal systems isolated from skin have provided useful information on skin biotransformation, there has been a need for more useful experimental models to examine cutaneous metabolism. The isolated perfused porcine skin flap (IPPSF) has been shown to represent a viable, fully functional, and predictive *in vitro* model system (*104,105*). Drugs, pesticides, and other xenobiotics that are applied topically to the IPPSF diffuse through the layers of the skin, and the chemical and its metabolites are taken up by the microcirculation, which closely mimics *in vivo* conditions. In addition, the test chemical may be administered intra-arterially to examine the fate of chemicals that distribute to the skin from the systemic circulation. We have used this model to study the metabolism and disposition of topically applied parathion (*105*). Parathion was applied to the surface of skin flaps representing three treatment groups: control, occluded, and 1-aminobenzotriazole (ABT)-pretreated. Radiolabel uptake in the perfusion medium indicated that total chemical flux and peak rates of absorption in occluded preparations were 59% and 47% lower than in controls, respectively, and both were >75% lower in the ABT flaps. Most of the absorbed radiolabel recovered in the perfusion medium of the control group was paraoxon (68%); there was a lesser amount of *p*-nitrophenol (15%), and the remainder was unmetabolized parathion. Occlusion of the application site increased the fraction of *p*-nitrophenol (43%) to that of paraoxon (40%) without altering the parent compound recovered. Pretreatment of the IPPSF with ABT (an inhibitor of P450) blocked most of the paraoxon formation (7%) but not that of *p*-nitrophenol (12%), while allowing 78% of the parathion absorbed to penetrate intact. These findings show that parathion undergoes significant biotransformation following topical application to porcine skin and that the resultant cutaneous metabolite profiles can be altered by both physical (occlusion) and chemical (ABT) means.

As indicated above in the case of mirex, pesticides passing through the skin can also be shown to affect the expression of P450 isozymes in the liver.

Studies in the laboratory of Mary Beth Genter (personal communication) using immunohistochemical methods have revealed that at least two isozymes of the FMO are present in the olfactory epithelium. FMO3 is broadly distributed while FMO1 has a more restricted distribution.

Future Studies

It appears clear that future epidemiology studies on the effects of pesticides on

human health must be more extensive if significance is to be properly assessed. Companion toxicological studies will be necessary to provide a mechanistic basis for the correlations observed. Similarly, exposure assessment will need to be continued. In both cases, the occupationally exposed provide a useful pool to yield initial insights before the much more intractable problem of the general population is addressed.

The interactions of pesticides with P450, FMO, and other Phase I and Phase II enzymes have been studied for some time; however, isozyme specificity for metabolism, induction, and inhibition is still little understood. If toxicology is to serve the public interest by carrying out the kinds of studies that protect public health but, at the same time, allow efficient vector control and the production of food and fiber, a more holistic approach will be necessary. It is not sufficient to know whether an agricultural chemical is a substrate for microsomal oxidation. A number of other questions must be addressed: What are the specific enzymes and isoforms involved? Is the reaction an activation or a detoxication reaction? Does induction or inhibition occur? What is the relationship to other Phase I and Phase II enzymes? How are these relationships changed by other xenobiotics? What interactions occur at portals of entry and sites of toxic action? Can this information be integrated into risk assessments and provide a mechanistic rationale for epidemiological studies? Only with this type of fundamental information can applied problems in the field be addressed.

REFERENCES

1. Hodgson E, Levi PE. The flavin-containing monooxygenase (EC 1.14.13.8). In: Molecular Aspects of Monooxygenases and Bioactivation of Toxic Compounds (Arinc E, Schenkman JB, Hodgson E, eds). New York:Plenum Press, 1991;11–21.
2. Hodgson E, Levi PE. The role of the flavin-containing monooxygenase (EC 1.14.13.8) in the metabolism and mode of action of agricultural chemicals. Xenobiotica 22:1175–1183 (1992).
3. Levi PE, Hodgson E. Metabolism of organophosphorus compounds by the flavin-containing monooxygenase. In: Organophosphates: Chemistry, Fate and Effects (Chambers JE, Levi PE, eds). San Diego:Academic Press, 1990;141–154.
4. Hodgson E, Blake BL, Levi PE, Mailman RB, Lawton MP, Philpot RM, Genter MB. Flavin-containing monooxygenases: substrate specificity and complex metabolic pathways. In: Molecular Aspects of Oxidative Drug Metabolizing Enzymes: Their Significance in Environmental Toxicology, Chemical Carcinogenesis and Health (Arinc E, Schenkman JB, Hodgson E, eds). Berlin:Springer Verlag, 1995;225–235.
5. Hodgson E, Rose RL, Adams NH, Deamer NJ, Genter MB, Venkatesh K, Levi PE. The role of oxidative enzymes in the metabolism and toxicity of pesticides. In: Molecular Aspects of Oxidative Drug Metabolizing Enzymes: Their Significance in Environmental Toxicology, Chemical Carcinogenesis and Health (Arinc E, Schenkman JB, Hodgson E, eds). Berlin:Springer Verlag, 1995;461–478.
6. Hodgson E, Rose RL, Ryu D-Y, Falls G, Blake BL, Levi PE. Pesticide-metabolizing enzymes. Toxicol Lett (in press).
7. Hodgson E, Silver IS, Butler LE, Lawton MP, Levi P. Metabolism. In: Handbook of Pesticide Toxicology, Vol 1. General Principles (Hayes WJ Jr, Laws ER Jr, eds). San Diego:Academic Press, 1991;107–167.
8. Kulkarni AP, Hodgson E. Metabolism of insecticides by mixed-function oxidase systems. Pharmacol Therap 8:379–475 (1980).
9. Nelson DR, Kamataki T, Waxman DJ, Guengerich FP, Estabrook RW, Feyereisen R, Gonzalex FJ, Coon MJ, Gunsalus IC, Gotoh O, Okuda K, Nebert DW. The P450 superfamily: update on new sequences, gene mapping, accession numbers, early trivial names of enzymes and nomenclature. DNA Cell Biol 12:1–51 (1993).
10. Ziegler DM, Mitchell CH. Microsomal oxidase. IV. Properties of a mixed-function amine oxidase isolated from pig liver microsomes. Arch Biochem Biophys 150:116–125 (1972).
11. Ziegler DM. Flavin-containing monooxygenases: enzymes adapted for multisubstrate specificity. Trends Pharmacol Sci 11:321–324 (1990).
12. Ziegler DM. Unique properties of the enzymes of detoxication. Drug Metabol Disp 19:847–852 (1991).
13. Ferrer A, Cabral R. Collective poisonings caused by pesticides: mechanisms of production mechanisms of prevention. Rev Environ Toxicol 5:161–201 (1993).
14. Rosenstock L, Keifer M, Daniell WE, McConnell R, Claypoole K. Chronic central nervous system effects of acute organophosphate pesticide intoxication. The Pesticide Health Effects Study Group. Lancet 338:223–227 (1991).
15. Baker SR, Wilkinson CF. The effects of pesticides on human health. Princeton, NJ: Princeton Scientific Publishing, 1990.
16. Blair A. A review of epidemiological studies of agriculture exposures and cancer. In: Proceedings of Agricultural, Occupational and Environmental Health: Policy Strategies for the Future (Donham KJ, Merchant JA, Kross B, eds). Am J Indust Med 1990;18:91–113.
17. Blair A, Zahm SH. Cancer among farmers. In: Occupational Medicine: State of the Art Reviews (Cordes DH, Rea DF, eds). Philadelphia:Hanley and Belfus, 1991;201–214.
18. Blair A, Dosemeci M, Heineman EF. Cancer and other causes of death among male and female farmers from twenty-three states. Am J Ind Med 23:729–742 (1993).
19. Blair A, Malker H, Cantor K, Burmeister L, Wiklund K. Cancer among farmers: a review. Scand J Work Environ Health. ll:397–407 (1985).
20. Brown LM, Blair A, Gibson R, Everett GD, Cantor KP, Schuman LM, Burmeister LF, Van Lier SF, Dick F. Pesticide exposures and other agricultural risk factors for leukemia among farmers in Iowa and Minnesota. Cancer Res 50:6585–6591 (1990).
21. Wolff M, Toniolo PG, Lee E, Rivera M, Dubin, N. Blood levels of organochlorine residues and risk of breast cancer. J Natl Cancer Inst 85:648–652 (1993).
22. Krieger N, Wolff MS, Hiatt RA, Rivera M, Vogelman J, Orentreich N. Breast cancer and serum organochlorines: a perspective study among white, black and Asian women. J Natl Cancer Inst 88(8):589–599 (1994).
23. Stone R. Environmental estrogens stir debate. Science 265:308–310 (1994).
24. Davis DL, Bradlow HL, Wolff M, Woodruff T, Hoel DG, Anton-Culver H. Medical hypothesis: xenoestrogens as preventable causes of breast cancer. Environ Health Perspect 101:372–377 (1993).
25. Colborn T, von Saal FS, Soto AM. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect 101(5):378–384 (1993).
26. Moser GJ, Meyer SA, Smart RC. The chlorinated pesticide mirex is a novel nonphorbol ester-type tumor promoter in mouse skin. Cancer Res 52:631–636 (1992).
27. Moser GJ, Robinette CL, Smart RC. Characterization of skin tumor promotion by mirex: structure-activity relationships, sexual dimorphism and presence of Ha-*ras* mutation. Carcinogenesis 14(6)1155–1160 (1993).
28. Meyer SA, Moser GJ, Monteiro-Riviere NA, Smart RC. Minimal role of enhanced cell proliferation in skin tumor promotion by mirex: a nonphorbol ester-type promoter. Environ Health Perspect 101(Suppl 5):265–270 (1993).

29. Meyer SA, Kim TW, Moser GJ, Monteiro-Riviere NA, Smart RC. Synergistic interaction between the non-phorbol ester-type promoter mirex and 12-*O*-tetradecanoylphorbol-13-acetate in mouse skin tumor promotion. Carcinogenesis 15:47–52 (1994).
30. Ecobichon DJ, Davis JE, Doull J, Ehrich M, Joy R, McMillan D, MacPhail R, Reiter LW, Slikker, W, Tilson, H. Neurotoxic effects of pesticides. In: The Effects of Pesticides on Human Health (Baker SR, Wilkinson CF, eds). Princeton, NJ:Princeton Scientific Publishing, 1990;131–199.
31. Tanner RW, Langston JW. Do environmental toxins cause Parkinson's disease? A critical review. Neurology 40:17–30 (1990).
32. Gordon JE, Shy CM. Agricultural chemical use and congenital cleft lip and/or palate. Arch Environ Health 36:213–220 (1981).
33. Mattison DR, Bogumil RJ, Chapin, R, Hatch M, Hendrickx A, Jarrell J, LaBarbera AL, Schrader SM, Seleval S. Reproductive effects of pesticides. In: The Effects of Pesticides on Human Health (Baker SR, Wilkinson CF, eds). Princeton, NJ: Princeton Scientific Publishing, 1990;297–389.
34. Gray LE Jr, Ostby J, Ferrell J, Sigmon R, Cooper R, Goldman J, Slott V, Laskey J. A dose–response analysis of methoxychlor-induced alterations of reproductive development and function in the rat. Fundam Appl Toxicol 12:92–108 (1989).
35. Gray LE. Chemical-induced alterations of sexual differentiation: a review of effects in humans and rodents. In: Chemically-Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection (Colborn T, Clement C, eds). Princeton, NJ:Princeton Scientific Publishing 1992;203–230.
36. Gray LE Jr, Ostby JS, Kelce WR. Developmental effects of an environmental antiandrogen: the fungicide vinclozolin alters sex differentiation of the male rat. Toxicol Appl Pharmacol 129:46–52 (1994).
37. Hileman B. Environmental estrogens linked to reproductive abnormalities and cancer. Chem Eng News 1994:Jan 31, 19–23 (1994).
38. Kelce WR, Stone CR, Laws SC, Gray LE, Kemppainen JA, Wilson EM. Persistent DDT metabolite *p,p'*-DDE is a potent androgen receptor antagonist. Nature 375:581–582 (1995).
39. Gray LE, Ostby J, Ferrell J, Sigmon R, Cooper R, Goldman J, Slott V, Laskey J. A dose–response analysis of methoxychlor-induced alterations of reproductive development and function in the rat. Fundam Appl Toxicol 12:92–108 (1989).
40. Gray LE. Chemical-induced alterations of sexual differentiation: a review of effects in humans and rodents. In: Chemically-Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection (Colborn T and Clement C, eds). Princeton, NJ: Princeton Scientific Publishers, 1992;203–230.
41. Gray LE, Ostby JS, Kelce WR. Developmental effects of an environmental antiandrogen: the fungicide vinclozolin alters sex differentiation of the male rat. Toxicol Appl Pharmacol 129:46–52 (1994).
42. Tynes RE, Hodgson E. Magnitude of involvement of the mammalian flavin-containing monooxygenase in the microsomal oxidation of pesticides. J Agric Food Chem 33:471–479 (1985).
43. Smyser BP, Sabourin PJ, Hodgson E. Oxidation of pesticides by purified microsomal FAD-containing monooxygenase from mouse and pig liver. Pestic Biochem Physiol 24:368–374 (1985).
44. Venkatesh K, Levi PE, Inman AO, Monteiro-Riviere NA, Misra R, Hodgson E. Enzymatic and immunohistochemical studies on the role of cytochrome P450 and the flavin-containing monooxygenase of mouse skin in the metabolism of pesticides and other xenobiotics. Pestic Biochem Physiol 43:53–66 (1992).
45. Levi PE, Hodgson E. Stereospecificity in the oxidation of phorate and phorate sulphoxide by purified FAD-containing monooxygenase and cytochrome P450 isozymes. Xenobiotica 18:29–39 (1988).
46. Levi PE, Hodgson E. Oxidation of pesticides by purified cytochrome P450 isozymes from mouse liver. Toxicol Lett 24:221–228 (1985).
47. Beumel GA, Levi PE, Hodgson E. Spectral interactions of piperonyl butoxide and isocyanides with purified hepatic cytochrome P450 from uninduced mice. Gen Pharmacol 16:193–197 (1985).
48. Levi PE, Hollingworth RM, Hodgson E. Differences in oxidative dearylation and desulfuration of fenitrothion by cytochrome P450 isozymes and in the subsequent inhibition of monooxygenase activity. Pestic Biochem Physiol 32:224–231 (1988).
49. Kinsler S, Levi PE, Hodgson E. Hepatic and extrahepatic microsomal oxidation of phorate by the cytochrome P450 and FAD-containing monooxygenase systems in the mouse. Pestic Biochem Physiol 31:54–60 (1988).
50. Kinsler S, Levi PE, Hodgson E. Relative contributions of the cytochrome P450 and flavin-containing monooxygenases to the microsomal oxidation of phorate following treatment of mice with phenobarbital, hydrocorisone, acetone and piperonyl butoxide. Pestic Biochem Physiol 23:174–181 (1990).
51. Chambers JE, Chambers HW. Oxidative desulfuration of chlorpyrifos, chlorpyrifos-methyl and leptophos by rat brain and liver. J Biochem Toxicol 4:201–203 (1989).
52. Kolmodin B, Azarnoff DL, Sjöquist F. Effect of environmental factors on drug metabolism: decreased plasma half-life of antipyrine in workers exposed to chlorinated hydrocarbon insecticdes. Clin Pharmacol Ther 10:638–642 (1969).
53. Kolmodin-Hedman B. Decreased plasma half-life of phenylbutazone in workers exposed to chlorinated pesticides. Eur J Clin Pharmacol 5:195–198 (1973).
54. Poland AP, Smith D, Kuntzman R, Jacobson M, Conney AH. Effect of intensive occupational exposure to DDT on phenyl butazone and cortisol metabolism in human subjects. Clin Pharmacol Ther 11:724–732 (1970).
55. Guzelian PS, Vramian G, Boylan JJ, Cohn WJ, Blake RV. Liver structure and function in patients poisoned with chlordecone. Gastroenterology 78:206–213 (1980).
56. Kreiss K, Zack MM, Kimbrough RD, Needham LL, Smrek AL, Jones BT. Cross-sectional study of a community with exceptional exposure to DDT. JAMA 245:1926–1930 (1981).
57. Abernathy CO, Hodgson E, Guthrie FE. Structure–activity relationships on the induction of hepatic microsomal enzymes in the mouse by 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl ethane (DDT) analogs. Biochem Pharmacol 20:2385–2393 (1971).
58. Abernathy CO, Philpot RM, Guthrie FE, Hodgson E. Inductive effects of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl ethane (DDT), phenobarbital, and benzyprene on microsomal P_{450}, ethyl isocyanide spectra, and metabolism *in vivo* of zoxazolamine and hexobarbital in the mouse. Biochem Pharmacol 20:2395–2400 (1971).
59. Lewandowski M, Levi PE, Hodgson E. Induction of cytochrome P450 isozymes by mirex and chlordecone. J Biochem Toxicol 4:195–199 (1989).
60. Hodgson E, Philpot RM. Intraction of methylenedioxyphenyl (1,3-benzodioxole) compounds with enzymes and their effects on mammals. Drug Metab Rev 3:231–301 (1974).
61. Goldstein JA, Hickman P, Kimbrough RD. Effects of purified and technical piperonyl butoxide on drug-metabolizing enzymes and ultrastructure of rat liver. Toxicol Appl Pharmacol 26:444–453 (1973).
62. Philpot RM, Hodgson E. The production and modification of cytochrome P-450 difference spectra by *in vivo* administration of methylenedioxyphenyl compounds. Chem Biol Interact 4:185–194 (1972).
63. Kamienski FX, Murphy SD. Biphasic effects of methylenedioxyphenyl synergists on the action of hexabarbital and organophosphate insecticides in mice. Toxicol Appl Pharmacol 18:883–894 (1971).
64. Dahl AR, Hodgson E. The interaction of aliphatic analogs of methylene-dioxyphenyl compounds with cytochromes P-450 and P-420. Chem Biol Interact 27:163–175 (1979).
65. Lewandowski M, Chui YC, Levi PE, Hodgson E. Differences in induction of hepatic cytochrome P450 isozymes in mice by eight methylenedioxyphenyl compounds. J Biochem Toxicol 5:47–55 (1990).
66. Adams NH, Levi PE, Hodgson E. Regulation of cytochrome P450 isozymes by methylenedioxyphenyl compounds. Chem Biol Interact 86:225–274 (1993).

67. Adams NH, Levi PE, Hodgson E. Differences in induction of three P450 isozymes by piperonyl butoxide, sesamex, safrole and isosafrole. Pestic Biochem Physiol 46:15–26 (1993)
68. Adams NH, Levi PE, Hodgson E. Regulation of cytochrome P450 isozymes by methylenedioxyphenyl compounds—an updated review of the literature. Rev Biochem Toxicol 11:205–222 (1995).
69. Cook JC, Hodgson E. The induction of cytochrome P450 by isosafrole and related methylenedioxyphenyl compounds. Chem Biol Interact 54:299–315 (1985).
70. Cook JC, Hodgson E. Induction of cytochrome P450 in cogenic C57BL/6J mice by isosafrole: lack of cerrelation with the Ah locus. Chem Biol Interact 58:223–240 (1986).
71. Marcus CB, Wilson NM, Jefcoate CR, Wilkinson CF. Selective induction of cytochrome P450 isozymes in rat liver by 4-n-alkyl methylenedioxybenzenes. Arch Biochem Biophys 227:8–16 (1990).
72. Murray M, Wilkinson CF, Dube CE. Induction of rat hepatic microsomal cytochrome P450 and aryl hydrocarbon hydroxylase by 1,3-benzodioxole derivatives. Xenobiotica 15:361–369 (1985).
73. Ryu D-Y, Levi PE, Hodgson E. Regulation of P450 isozymes CYP1A1, CYP1A2 and CYP2B10 by three benzodioxole compounds. Chem Biol Interact 96:235–247 (1995).
74. Moody DE, Hammock BD. The effect of tridiphane (2-(3,5-dichlorophenyl)-2-(2,2,2- trichloroethyl)oxirane) on hepatic epoxide-metabolizing enzymes: indications of proxisome proliferation. Toxicol Appl Pharmacol 89:37–46 (1987).
75. Levi PE, Rose RL, Adams NH, Hodgson E. Induction of cytochrome P450 4A1 in mouse liver by the herbicide synergist tridiphane. Pestic Biochem Physiol 44:1–19 (1992).
76. Falls JG, Cao Y, Blake BL, Levi PE, Hodgson E. Gender differences in hepatic expression of flavin-containing monooxygenase isoforms (FMO1, FMO3 and FMO5) in CD-1 mice. J Biochem Tox 10(3):171–177 (1995).
77. Moreland DE, Novitsky WP, Levi PE. Selective inhibition of cytochrome P450 isozymes by the herbicide synergist tridiphane. Pestic Biochem Physiol 35:42–49 (1989).
78. Kamataki T, Neal RA. Metabolism of diethyl *p*-nitrophenyl phosphorothionate (parathion) by a reconstituted mixed-function oxidase enzyme system: studies of the covalent binding of the sulfur atom. Mol Pharmacol 12:933–944 (1976).
79. Halpert J, Hammond D, Neal RA. Inactivation of purified rat liver cytochrome P450 during the metabolism of parathion (diethyl *p*-nitrophenyl phosphorothionate). J Biol Chem 255:1080–1089 (1980).
80. Morelli MA, Nakatsugawa T. Inactivation *in vitro* of microsomal oxidases during parathion metabolism. Biochem Pharmacol 27:293–299 (1978).
81. Uchiyame M, Yoshida T, Homma K, Hongo T. Inhibition of hepatic drug-metabolizing enzymes by thiophosphate insecticides and its drug toxicological implications. Biochem Pharmacol 24:1221–1225 (1975).
82. Neal RA, Halpert J. Toxicology of thiono-sulfur compounds. Annu Rev Pharmacol Toxicol 22:321–339 (1982).
83. Neal RA, Swahata T, Halpert J, Kametaki T. Chemically reactive metabolism as suicide enzyme inhibitors. Drug Metabol Rev 14:49–59 (1983).
84. Neal RA. Thiono-sulfur compounds. In: Bioactivation of Foreign Compounds (Anders MW, ed). New York:Academic Press, 1985;519–540.
85. Tynes RE, Hodgson E. Oxidation of thiobenzamide by the FAD-containing and cytochrome P450-dependent monooxygenases of liver and lung microsomes. Biochem Pharmacol 32:3419–3428 (1983).
86. Blake BL, Rose RL, Mailman RB, Levi PE, Hodgson E. Metabolism of thioridazine by microsomal monooxygeanses: relative roles of P450 and flavin-containing monooxygenase. Xenobiotica 25:377–393 (1995).
87. Hajjar NP, Hodgson E. The microsomal FAD-dependent monooxygenase as an activating enzyme: fonofos metabolism. In: Biological Reactive Intermediates II, Part B (Synder, Parke, Kocsis, Jollow, Gibson,Witmer, eds). New York:Plenum Publishing, 1982:1245–1253.
88. Smyser BP, Levi PE, Hodgson E. Interactions of diethylphenylphosphine with purified, reconstituted mouse liver cytochrome P450 monooxygenase systems. Biochem Pharmacol 35:(10)1719–1723 (1986).
89. Lawton MP, Philpot RM. Molecular genetics of the flavin-containing monooxygenases. Pharmacogenetics 3:40–44 (1993).
90. Lawton MP, Philpot RM. Emergence of the flavin-containing monooxygenase gene family. Rev Biochem Toxicol 11:1–27 (1995).
91. Lawton MP, Cashman JR, Cresteil T, Dolphin CT, Elfarra AA, Hines RN, Hodgson E, Kimura T, Ozols J, Phillips IR, Philpot RM, Poulsen LL, Retti AE, Shephard EA, Williams DE, Ziegler DM. A nomenclature for the mammalian flavin-containing monooxygenase gene family based on amino acid sequence identities. Arch Biochem Biophys 308:254–257 (1994).
92. Dolphin C, Shepard EA, Povey S, Palmer CN, Zeigler DM. Cloning, primary sequence and chromosome mapping of a human flavin-containing monooxygenase (FMO). J Biol Chem 266:12379–12385 (1991).
93. Veechini F, Michel S. Importance of cytochrome P450 for the development of new drug concepts in the skin. Eur J Dermatol 4:583–588 (1994).
94. Mukhtar H, Bickers DR. Drug metabolism in skin. Comparative activity of the mixed-function oxidases, epoxide hydratase, and glutathione *S*-transferase in liver and skin of the neonatal rat. Drug Metab Dispos 9:311–314 (1981).
95. Kao J, Carver MJ. Cutaneous metabolism of xenobiotics. Drug Metab Rev 22:363–410 (1990).
96. Rettie AE, Williams FM, Rawlins MD. Substrate specificity of the mouse skin mixed-function oxidase system. Xenobiotica 16:205–211 (1986).
97. Finnen MJ. Skin metabolism by oxidation and conjugation. Pharmacol Skin 1:163–174 (1987).
98. Storm JE, Collier SW, Stewart RF, Bronaugh RL. Metabolism of xenobiotics during percutaenous penetration: role of absorption rate and cutaneous enzyme activity. Fundam Appl Toxicol 15:132–141 (1990).
99. Khan WA, Park SS, Gelboin HV, Bickers DR, Mukhtar H. Epidermal cytochrome P450: immunochemical characterization of isoform induced by topical application of 3-methylcholanthrene to neonatal rat. J Pharmacol Exp Ther 249:921–927 (1989).
100. Khan WA, Park SS, Gelboin HV, Bickers DR, Mukhtar H. Monoclonal antibodies directed characterization of epidermal and hepatic cytochrome P-450 isozymes induced by skin application of therapeutic crude coal tar. J Invest Dermatol 93:40–45 (1989).
101. Bickers DR, Wroblewski D, Dutta-Choudhury T, Mukhtar H. Induction of neonatal rat skin and liver aryl hydrocarbon hydroxylase by coal tar and its constitutents. J Invest Dermatol 78:227–229 (1982).
102. Jugert FK, Agarwal R, Kuhn A, Bickers DR, Merk HF, Mukhtar H. Multiple cytochrome P450 isozymes in murine skin: induction of P450 1A, 2B, 2E, and 3A by dexamethasone. J Invest Dermatol 102:970–975 (1994).
103. Goerz G, Bolsen K, Kalofoutis A, Tsambaos D. Influence of oral isotretinoin on hepatic and cutaneous P450-dependent isozyme activities. Arch Dermatol Res 286:104–106 (1994).
104. Riviere JE, Bowman KF, Monteiro-Riviere NA, Dix LP, Carver MP. The isolated perfused porcine skin flap (IPPSF). Fundam Appl Toxicol 7:444–453 (1986).
105. Carver MP, Levi PE, Riviere JE. Parathion metabolism during percutaenous absorption in perfused porcine skin. Pestic Biochem Physiol 38:245–254 (1990).